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The Effect of the Dexamethsone on Sperm Characteristic and Testosterone Level on Awassi Rams

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Abstract: The aim of this study, was to determine the effects of dexamethasone on sperm characteristics (volume, mass motility, individual motility, sperm concentration, osmotic pressure) and testosterone activity of serum. In this study, 9 healthy Awassi rams, at the age of 3-5 years and weighing between 50-60 kg, were used. The rams were randomly divided into 2 groups (4 in control group and 5 in treatment group). Dexamethasone was administered intramuscularly at a dose of 4 mg ram^{-1} once weekly for 3 weeks, semen samples were collected from the rams 2, 24 and 48 h after each injection weekly and examined for sperm characteristics. The results showed that the use of dexamethasone caused significant ($p<0.05$) increase in semen volume, mass motility and individual sperm motility as compared to the control group. There was no significant correlation ($p<0.05$) in semen osmotic pressure and sperm concentration. Blood samples were collected from the ram before treatment, after 10 and 25 days of treatment and examined for serum testosterone. The result showed there are significant difference ($p<0.05$) after 25 days after treatment when compared with the control group.

Key words: Effect, dexamethsone, sperm characteristic, testosterone, awassi, rams

INTRODUCTION

Adrenal cortex secretes a number of corticosteroids, classified into glucocorticoids like Cortisol, mineralocorticoids like aldosteron and gonadocorticoids like androgen (Reynolds, 1996).

Corticosteroids used for medical purpose for >40 years (Zoorob and Cender, 1998). One of this corticosteroids is glucocorticoids, which used its therapeutic doses as anti-inflammatory, immunosuppressive and many other uses (Wilder, 2001) and glucocorticoids present naturally in the body like Cortisol and some of it synthetic like Dexamethasone (Nelson and Cox, 2005) and used largely as anti-inflammatory.

Glucocorticoids synthesis in zona fasiculata and zona reticularies layers of adrenal cortex (Chrousos and Margiors, 2001). Dexamethasone is synthetic glucocorticoid (Van, 1994).

Dexamethasone used largely in treatments of human being and animals (Donald, 2002) and used in treatment hematological disorders, leukemia and cerebral edema (Laurence *et al*., 1997) (Reynolds, 1996), nephritic syndrome and subacute thyroiditis (Williams and Dluhy, 1998), allergic reactions, chronic hepatitis, sub acute hepatic necrosis, rheumatoid arthritis, asthma, chronic pulmonary disease, transplanted organs rejection and malignant neoplasm (Chrousos and Margiors, 2001), eyes disease like conjunctivitis, dermatitis and eczema (Rang *et al*., 2003; Donald, 2002).

In a recent study Michelle *et al*. (2004), pregnant ewes were treated with dexamethasone, treatment cases a significant decreased in fetal weight, fetal brain weight and prolong the gestation periods.

The aim of this study, was to determine the effects of dexamethasone on sperm characteristics (volume, mass motility, individual motility, sperm concentration, osmotic pressure) and testosterone activity of serum.

MATERIALS AND METHODS

Animals and drugs administration: The experiment was carried out during the months May and June (non breeding season) 2008. Nine adult Awassi rams (3-5 years old) with an average 57±5 kg body weight were used in this study. All rams had been reared under similar conditions (they were fed on hay grain diet and had free access to water), the rams housed in the animals house of the Veterinary Medicine college, University of Mosul, Mosul, Iraq.

The rams were divided randomly into 2 groups. This groups was assigned as a control ($n = 4$) and treatment ($n = 5$). Serum testosterone and sperm characteristics of all rams in each group were determined prior to drug injection.

Dexamethasone (colvasone-Norbrook) was administered intramuscularly at a dose of 4 mg ram^{-1} once weekly for 3 weeks.

Semen samples were taken by using artificial vagina from all rams at 2.24 and 48 h. Semen volume was determined by direct reading the graduation of collection tubes (0.1-10 mL). Sperm concentration was determined by a spectrophotometer (Sherwood colorimeter 254). Mass motility determinate by direct drop on warm slide, a slide was placed on phase contrast microscope (Gund lach) and allowed to warm up to 37°C and then a small droplet of semen was placed on the slide and percent motility was evaluated visually at a magnification of 4×.

Individual motility determinate, Semen were decimally diluted with isotonic sodium citrate solution at 37°C (2.9% $w\ v^{-1}$, dissolved in distilled water) at the rate of 1:10. A slide was placed on phase contrast microscope and allowed to warm up to 37°C and then a small droplet of diluted semen was placed on the slide and percent motility was evaluated visually at a magnification of 40×.

Motility estimations were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score (Bearden and Fuquay, 2000).

Osmotic pressure of semen determined in seminal plasma by centrifugation plasma semen samples in 2000 rpm and take the seminal plasma and test it in (gonotec osmomat 030).

Blood samples: About 5 mL of blood were collected from jugular vein from all rams (control and treatment groups) before treatment, after 10 days of treatment and after 25 days of treatment and examined for serum testosterone Assays. The blood samples were allowed to clot at refrigerator, after centrifugation (2000 rpm for 15 min) the serum was separated and stored at -18 to -20°C until analysis was taken. Serum testosterone level were determined by the using of Enzyme Linked Immuno Sorbent Assay (ELISA) (Hollandstr. 17, D-53881 Euskirchen, Germany) (Bearden *et al*., 2004).

Statistical analysis: The results were expressed as mean±SEM. Data were analyzed statistically using tow way Analysis of Variance (ANOVA), with Duncan's Multiple Range by Sigma Stat soft were (Petrie and Watson, 1999).

RESULTS

The difference in semen volume showed in Table 1. The results showed an non significant increased in semen volume between treatment and control groups, only groups 4 and 8 are significant difference ($p<0.05$).

The difference in sperm mass motility showed in Table 2. The results showed an non significant increased in sperm mass motility between treatment and control groups.

Table 1: Effect of dexamethasone treatment on awassi ram semen volume (mL) (mean±SE)

	1st week		
	2 h	24 h	48 h
Collection	1	2	3
Control	0.725±0.157	0.625±0.157	0.650±0.157
Treatment	0.740±0.140	1.340±0.140	0.830±0.140d
	2nd week		
	2 h	24 h	48 h
Collection	4	5	6
Control	0.900±0.157	0.900±0.157	0.813±0.157
Treatment	1.040±0.140b*	1.220±0.140e	1.280±0.140
	3rd week		
	2 h	24 h	48 h
Collection	7	8	9
Control	0.850±0.157	0.750±0.157	0.850±0.157
Treatment	1.250±0.140c	1.000±0.140*a,c*	0.940±0.140a,b,d,e

Table 2: Effect of dexamethasone treatment on awassi ram sperm mass motility (%) (mean±SE)

	1st week		
	2 h	24 h	48 h
Collection	1	2	3
Control	71.250±3.027	81.250±3.027	78.750±3.027
Treatment	78.000±2.707	81.000±2.707a-c	79.000±2.707
	2nd week		
	2 h	24 h	48 h
Collection	4	5	6
Control	72.500±3.027	77.500±3.027	72.500±3.027
Treatment	81.000±2.707c	79.000±2.707	78.000±2.707
	3rd week		
	2 h	24 h	48 h
Collection	7	8	9
Control	78.750±3.027	80.000±3.027	78.750±3.027
Treatment	80.000±2.707a	90.000±2.707	85.000±2.707b

Different letters mean there are significant difference ($p<0.05$), *:mean there are significantly different ($p<0.05$) within the groups

The difference in sperm individual motility showed in Table 3. The results showed an non significant increased in sperm individual motility between treatment and control groups, only groups 2 and 6 are significant difference ($p<0.05$) and group 7 showed non significant decreased.

The difference in semen serum osmotic pressure shown in Table 4. The results showed an non significant increased in semen serum osmotic pressure between treatment and control groups, only groups 4 and 6 are significant difference ($p<0.05$) and group 8 showed non significant decreased.

Table 3: Effect of dexamethasone treatment on awassi ram semen individual motility (%) (mean±SE)

	1st week		
	2 h	24 h	48 h
Collection	1	2	3
Control	76.25±3.447	82.50±3.447	77.50±3.447
Treatment	83.00±3.083e,f	84.00±3.083a-d,*	80.00±3.083

	2nd week		
	2 h	24 h	48 h
Collection	4	5	6
Control	77.50±3.447	83.75±3.447	75.00±3.447
Treatment	88.00±3.083b,f	86.00±3.083d	85.00±3.083c*

	3rd week		
	2 h	24 h	48 h
Collection	7	8	9
Control	82.50±3.447	86.25±3.447	81.25±3.447
Treatment	80.00±3.083	90.00±3.083	84.00±3.083a,e

Table 4: Effect of dexamethasone treatment on awassi ram semen serum osmotic pressure (mean±SE)

	1st week		
	2 h	24 h	48 h
Collection	1	2	3
Control	387.0±15.701	398.750±15.701	430.250±15.701
Treatment	408.200±14.043	414.600±14.043a	448.200±14.043b

	2nd week		
	2 h	24 h	48 h
Collection	4	5	6
Control	392.250±15.701	401.750±15.701	398.500±15.701
Treatment	410.000±14.043*	410.000±14.043	431.800±14.043d*

	3rd week		
	2 h	24 h	48 h
Collection	7	8	9
Control	385.500±15.701	390.000±15.701	415.000±15.701
Treatment	421.000±14.043a-d	380.800±14.043	414.800±14.043c

Different letters mean there are significant difference ($p<0.05$), *:mean there are significantly different ($p<0.05$) within the groups

The difference in semen sperm concentration showed in Table 5. The results showed an no correlation in sperm concentration between treatment and control groups.

The difference in serum testosterone level showed in Table 6. The results showed an significant correlation in serum testosterone between before treatment and 25 days after treatment, but no significant correlation between before treatment and 10 days after treatment.

Table 5: Effect of dexamethasone treatment on awassi ram semen sperm concentration (X10^6) (mean±SE)

	1st week		
	2 h	24 h	48 h
Collection	1	2	3
Control	3422.350±317.225	3359.500±317.225	3422.350±317.225
Treatment	3319.260±283.735	3364.520±283.735	3605.860±283.735

	2nd week		
	2 h	24 h	48 h
Collection	4	5	6
Control	3233.790±317.225	3485.188±317.225	2649.225±317.225
Treatment	2831.520±283.735	2932.040±283.735	2901.920±283.735

	3rd week		
	2 h	24 h	48 h
Collection	7	8	9
Control	3359.450±317.225	3359.475±317.225	3786.875±317.225
Treatment	2846.560±283.735	3676.240±283.735	3454.878±283.735

Table 6: Effect of dexamethasone treatment on awassi ram serum testosterone level (ng mL^{-1}) (mean±SE)

Collection	Before treatment	10 days after treatment	25 days after treatment
Control	8.550±0.261	8.750±0.261	8.875±0.261
Treatment	10.000±0.261*	8.240±0.261	7.700±0.261*

*: Mean there are significantly different ($p<0.05$) within the groups

DISCUSSION

Dexamethasone treatment in this study showed there is some effect on ram semen characteristic. It has been shawn that there was an increase in volume, mass motility and individual motility, but there are no noticeable effect on sperm concentration and serum blood testosterone level.

In study by Tsantarliotou *et al.* (2002), showed that Dexamethasone induced a reduction in mean value and basal level of blood testosterone and inhibited its episodic secretion between 1 and 4 days after administration. As the reduction of acrosin activity appeared relatively soon after dexamethasone administration (7th day), it is likely that the increased amount of dexamethasone did not influence the synthesis of proacrosin in the late spermatids. As glucocorticoid receptors exist in the epididymis and accessory glands in various species, dexamethasone may have a direct influence on the synthesis and/or release of acrosin inhibitors in epididymal fluid or seminal plasma. These changes in acrosin activity in ovine spermatozoa mediated by dexamethasone may be of importance regarding the role of stress in the reduction of sperm fertilizing ability

(Tsantarliotou *et al*., 2002). In other study submeted by Gur *et al*. (2005) showed that dexamethasone increases hyaluronidase activity of serum and semen, but it decreases sperm concentration, semen volume and sperm motility in rams. Therefore, the use of these drugs in breeding rams during breeding season is not suitable (Gur *et al*., 2005). It was concluded that it restraint stress increased cortisol and decreased testosterone with minimal change in LH in sexually inactive and sexually active female and male-oriented rams, thus not providing a method to differentiate between ram classes (Stellflug, 2006).

The study on gilts by Otten *et al*. (2004) see that in gilts the adrenocortical response to an exogenous application of Synacthen® Depot is consistent over time during mid-gestation. Furthermore, cortisol but not ACTH levels were increased in fetuses from ACTH-treated sows, indicating that maternal cortisol can cross the placenta during mid-gestation. The stimulation of maternal cortisol release through exogenous ACTH with subsequent elevation of fetal cortisol levels is, therefore, a useful approach for studying effects of elevated maternal glucocorticoids in prenatal stress studies in pigs (Otten *et al*., 2004). And a single treatment of Vit E and Se at 3-week prepartum reduced concentrations of plasma cortisol and erythrocyte peroxide. Altered enzyme activities in the fetal membranes indicated the involvement of leukocytes and trauma at the fetomaternal junction and warrant further investigation (Gupta *et al*., 2005).

CONCLUSION

We concluded that dexamethasone increases semen volume, mass motility and individual sperm motility.

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